EFFECT UPON THE ANALGESIC ACTION OF RESERPINE OF CENTRAL NERVOUS SYSTEM STIMULANTS AND DRUGS AFFECTING THE METABOLISM OF CATECHOL- AND INDOLE-AMINES

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The effect upon the analgesic action of reserpine of central nervous system stimulants and of drugs affecting the metabolism of catechol- and indole-amines as measured in mice by a hot-plate method has been analysed. The analgesic effect, which has a maximal intensity 48 hr. after injection of the alkaloid, is partially or totally counteracted by MAO inhibitors, LSD-25 and 5-hydroxytryptophan. Central nervous system stimulants given to reserpinised mice before each test were effective in reducing the reaction time to the heat stimulation only 24 and 48 hr. after reserpine. DOPA showed no significant ability to reduce the analgesic effect of reserpine. In the light of these findings a suggestion has been made that 5-hydroxytryptamine rather than catecholamines would be involved in the mechanism of the analgesic effect due to reserpine and since central nervous system stimulants are able to reduce this action initially but are ineffective later, part of the effect is thought to be due to sedation of the animals, the participation of the stimulants being confined to a direct antagonism against the sedation. The lasting analgesic effect is supposed to correspond to a lack of 5-hydroxytryptamine in the cerebral structures.

IN a previous publication (Leme and Rocha e Silva, 1961) we described experiments in mice which showed increased reaction time (RT), under the influence of reserpine, to exposure to a hot-plate at 55°. This prolongation of the RT developed slowly, being maximal 48 hr. after the injection of reserpine, and returning slowly to normal levels 5–6 days after treatment. That this effect was due to a central analgesic effect was supported by the observation that reserpine potentiates morphine 2 hr. after their injection, while 24 to 48 hr. after reserpine its effects sum with those of morphine. The analgesic effect of reserpine differs from that of morphine, in being of slow onset and persistent for days, while that of morphine develops quickly and disappears after 1–2 hr. Since reserpine is known to deplete tissues of their catechol- and indole-amines (Pletscher, Shore and Brodie, 1956; Paasonen and Vogt, 1956), it seemed logical to assume that its analgesic effect might be related to its amine-releasing activity in the central nervous system. An interesting coincidence is the similar times needed for the replenishment of catechol- and indole-amines in the central nervous system after depletion by reserpine, observed in rabbits by Pletscher, Shore and Brodie (1956), and the return to normal of the RT in mice submitted to thermal stimulus.

We have now attempted to amplify our knowledge of the possible mechanism of the analgesic action of reserpine by analysing the action of compounds which might interfere with the metabolism of 5-hydroxytryptamine (5-HT) and catecholamines, such as inhibitors of monoamine

oxidase (MAO), 5-hydroxytryptophan (5-HTP), 3,4-dihydroxyphenylalanine (DOPA), LSD–25 and other central nervous system stimulants, such as sympathomimetic amines or bemegride.

MATERIALS AND METHODS

Male white mice, 15 to 20 g., were tested by the hot-plate (55°) method (Leme and Rocha e Silva, 1961). The RT was measured from the moment the animal was placed on the plate until it presented a sign of discomfort characterised by a licking of both front paws simultaneously or a sudden jump.

Solutions in distilled water were prepared in concentrations such that each animal received a maximum of 1 ml. injected intraperitoneally.

Reserpine was given in a single dose of 5 mg./kg. of the drug. The RT was measured 24, 48 and 72 hr. after the injection of reserpine in all instances.

Animals treated with MAO inhibitors and reserpine received two divided doses of the inhibitors 24 and 2 hr. before reserpine and the RT was measured as described.

Animals, previously injected with reserpine, received central nervous system stimulants in three doses, each given 30 min. before the tests for RT.

Mice treated with reserpine and LSD–25 were distributed into two groups: one received reserpine and a single dose of LSD–25 30 min. before the first exposure to the hot-plate; the other received reserpine and then a dose of LSD–25 was given 30 min. before each of the three RT tests.

Animals treated with 5-HTP or DOPA received a dose a day for 3 days before the reserpine. The RT was then measured as described.

A control group (no treatment) of 100 animals was exposed to the hot-plate three times at intervals of 24 hr. The RT had a mean $\pm$ s.e. of $10{\cdot}0 \pm 0{\cdot}4$ sec. in the first exposure, $11{\cdot}9 \pm 0{\cdot}5$ sec. in the second and $12{\cdot}3 \pm 0{\cdot}6$ in the third.

The following substances were used: reserpine, iproniazid, DL-trans-2-phenylcyclopropylamine (SKF385), pheniprazine, *N*-2-methyl-1,4-benzodioxane-*N*-benzylhydrazine tartrate (2596 IS), methylamphetamine hydrochloride, ephedrine sulphate, bemegride, D-lysergic acid diethylamide tartrate (LSD–25), DL-5-hydroxytryptophan, DL-3,4-dihydroxyphenylalanine.

RESULTS

Effect of reserpine on RT *at* 55°. We confirmed the previous results (Leme and Rocha e Silva, 1961) showing that reserpine in a single injection prolongs the RT for several days.

Ninety animals treated with reserpine showed an increase in RT (Table I) when compared with a control group (100 animals). The maximum increase is around 48 hr. after the injection of the drug, returning to normal after a few days. The observations were limited to 72 hr. after the injection.

Effect of MAO *inhibitors on* RT *at* 55° *of reserpine-treated mice.* All four MAO inhibitors, given before reserpine, counteracted partially or totally

TABLE I

REACTION TIMES OF A GROUP OF ANIMALS INJECTED WITH 5 MG./KG. RESERPINE, COMPARED WITH A CONTROL GROUP

Treatment	Reaction time (sec.)			
	24 hr. after reserpine (mean ± s.e.)	48 hr. after reserpine (mean ± s.e.)	72 hr. after reserpine (mean ± s.e.)	Number of animals
Reserpine: 5 mg./kg. (single dose) ..	16·9 ± 1·0	26·7 ± 1·3	18·2 ± 0·8	90
Controls (no treatment)	1st exposure 10·0 ± 0·4	2nd exposure 11·9 ± 0·5	3rd exposure 12·3 ± 0·6	100

the prolongation of RT produced by reserpine. Table II summarises the results.

Forty-five animals were given iproniazid, 200 mg./kg., in two equal doses 24 and 2 hr. before reserpine showed a significant reduction of RT.

Fifty animals received SKF385, 4 mg./kg., in two equal doses 24 and 2 hr. before reserpine showed a decrease in the RT mean values.

TABLE II

REACTION TIMES OF ANIMALS SUBMITTED TO COMBINED TREATMENT: MAO INHIBITORS + RESERPINE

Treatment before 5 mg./kg. reserpine	Reaction time (sec.)			
	24 hr. after reserpine (mean ± s.e.)	48 hr. after reserpine (mean ± s.e.)	72 hr. after reserpine (mean ± s.e.)	Number of animals
Iproniazid, total dose: 200 mg./kg. ..	12·5 ± 0·7	17·9 ± 0·9	14·8 ± 0·7	45
SKF 385, total dose: 4 mg./kg. ..	10·6 ± 0·5	14·6 ± 0·8	12·6 ± 0·6	50
Pheniprazine, total dose: 4 mg./kg. ..	9·7 ± 0·4	11·1 ± 0·5	9·6 ± 0·5	50
2596 IS, total dose: 20 mg./kg. ..	9·4 ± 0·4	10·7 ± 0·4	10·7 ± 0·5	50

Fifty animals were injected with two doses of pheniprazine, 2 mg./kg., 24 and 2 hr. before reserpine. This drug proved to be more potent than SKF385 (weight/weight) in reducing the RT mean values.

Two doses of 2596IS, 10 mg./kg., 24 and 2 hr. before reserpine in 50 animals were effective in reducing the RT mean values.

TABLE III

REACTION TIMES OF ANIMALS SUBMITTED TO COMBINED TREATMENT: RESERPINE + CNS STIMULANTS

Treatment	Reaction time (sec.)			
	24 hr. after reserpine (mean ± s.e.)	48 hr. after reserpine (mean ± s.e.)	72 hr. after reserpine (mean ± s.e.)	Number of animals
Reserpine: 5 mg./kg. + methyl-amphetamine: 3 doses (10 mg./kg./24 hr.)	15·1 ± 1·1	20·3 ± 1·2	16·7 ± 1·1	50
Reserpine: 5 mg./kg. + ephedrine: 3 doses (10 mg./kg./24 hr.)	12·0 ± 1·4	16·5 ± 1·0	16·3 ± 1·0	50
Reserpine: 5 mg./kg. + bemegride: 3 doses (1 mg./kg./24 hr.)	9·4 ± 0·7	16·6 ± 1·4	17·3 ± 1·6	50

Effect of central nervous system stimulants on RT *at* 55° *of reserpine-treated mice.* Methylamphetamine and ephedrine, 10 mg./kg., and bemegride, 1 mg./kg., injected 30 min. before the tests on the hot-plate in three groups of 50 animals previously treated with reserpine, caused a decrease in the mean values of RT, methylamphetamine being the least potent (Table III).

Effect of LSD–25 on RT *at* 55° *of reserpine-treated mice.* After reserpine, a single dose of 500 μg./kg. LSD–25, 30 min. before the first exposure to the plate, or three daily doses of 500 μg./kg. 30 min. before the RT tests, were effective in reducing the mean values of the RT. No significant difference was seen between the two groups (50 animals in each group) of mice. Thus, a single dose injected at the onset of the assay was as potent as three doses of the drug, given at 24 hr. intervals, decreasing the RT values of reserpine-treated mice, as seen in Table IV.

TABLE IV

REACTION TIMES OF MICE SUBMITTED TO COMBINED TREATMENT: RESERPINE + LSD–25

Treatment	Reaction time (sec.)			Number of animals
	24 hr. after reserpine (mean ± s.e.)	48 hr. after reserpine (mean ± s.e.)	72 hr. after reserpine (mean ± s.e.)	
Reserpine: 5 mg./kg. + LSD: one dose (500 μg./kg.)	12·6 ± 0·8*	16·0 ± 1·2	15·3 ± 1·0	50
Reserpine: 5 mg./kg. + LSD: 3 doses (500 μg./kg./24 hr.)	11·7 ± 0·8*	15·3 ± 1·0†	13·7 ± 0·9‡	50

* 45 min. after 1st dose LSD. † 45 min. after 2nd dose LSD. ‡ 45 min. after 3rd dose LSD.

Effect of 5-HTP *and* DOPA *on* RT *at* 55° *of reserpine-treated mice.* Three doses of 5-HTP, 100 mg./kg./24 hr., to 95 animals, before reserpine, reduced the RT values (Table V), while DOPA similarly injected into 75 animals showed no ability to reduce the RT prolonged by reserpine (Table V).

TABLE V

REACTION TIMES OF ANIMALS SUBMITTED TO COMBINED TREATMENT: 5-HTP OR DOPA AND RESERPINE

Treatment	Reaction time (sec.)			Number of animals
	24 hr. after reserpine (mean ± s.e.)	48 hr. after reserpine (mean ± s.e.)	72 hr. after reserpine (mean ± s.e.)	
5-HTP: 3 doses (100 mg./kg./24 hr.) + reserpine: 5 mg./kg.	13·5 ± 1·0	16·6 ± 1·0	14·3 ± 0·9	95
DOPA: 3 doses (100 mg./kg./24 hr.) + reserpine: 5 mg./kg.	20·9 ± 1·6	20·9 ± 1·3	20·2 ± 1·3	75

CONCLUSIONS AND DISCUSSION

Our results show that the analgesic action of reserpine, as measured in mice by a prolongation of RT when the animals are exposed to a hot-plate at 55°, is influenced by drugs given in association with the alkaloid.

The MAO inhibitors, iproniazid, SKF385, pheniprazine and 2596IS, decreased the RT values. No attempt has been made to relate the potency of these drugs to the analgesic action of reserpine.

The central nervous system stimulants were also able to reduce the analgesic effect. This reduction was observed 24 and 48 hr. after reserpine, but no significant reduction was seen 72 hr. after the alkaloid. Since methylamphetamine and ephedrine are also inhibitors of MAO (Gaddum and Kwiatkowski, 1938; Blaschko, 1952), at least part of their action could be due to this effect. However, bemegride which is devoid of such an action has been shown to be more potent in reducing RT after reserpine than the sympathomimetic amines.

LSD–25 displays a potent inhibitory effect upon the analgesic action of reserpine. No difference was observed when used as a single dose or with repeated daily doses.

5-HTP greatly inhibited the analgesic effect of reserpine, while DOPA had no action.

The analgesic action of reserpine as measured by the prolongation of the RT when mice are exposed to the hot-plate, shows peculiarities that might be discussed in the light of the above findings. The maximum effect is attained 48 hr. after the injection of the drug, and therefore might be interpreted as an indirect consequence of the action of the drug upon the physiological status of the reacting structures, either locally or in the central pathways for the painful stimuli. Since reserpine is known to deplete tissues of their stores of catechol- and indole-amines, the prolongation of the RT to heat stimulation, might be due to such a depletion in the central nervous system. In that case, the changes in RT observed after reserpine might reflect the degree of depletion of those amines in the brain. Interference by drugs affecting the metabolism of catechol- and indole-amines might give information about such a mechanism.

We have demonstrated this analgesic effect to be inhibited by MAO inhibitors. Though catechol-*O*-methyl transferase is concerned with the first step in a major metabolic pathway of adrenaline and noradrenaline (Axelrod, 1957; Axelrod and Tomchick, 1958), the inhibition of MAO has been demonstrated to effect a rise in the levels of these amines in tissues (Shore, Mead, Kuntzman, Spector and Brodie, 1957; Leroy and Schaepdryver, 1961). In a parallel way, the 5-HT content of brain tissues is increased when MAO inhibitors are administered to animals (Pletscher, Göschke, Gey and Thölen, 1961; Spector, Shore and Brodie, 1960). If these drugs are given before reserpine, there is less depletion of catechol- and indole-amines (Weil-Malherbe, Posner and Bowles, 1961; Brodie, Pletscher and Shore, 1956). That MAO inhibitors are effective in reducing the analgesic effect of reserpine could be taken as an indirect suggestion of the role played by catecholamines and 5-HT in this analgesic action.

5-HTP besides being a precursor of 5-HT, easily penetrates the blood-brain barrier. This drug was found to strongly reduce the analgesic action of reserpine. This could be additional evidence of the role of 5-HT in this action.

As far as the participation of catecholamines is concerned, DOPA, the

precursor of these amines, which behaves like 5-HTP in relation to the blood-brain barrier, did not reduce significantly the RT of reserpine-treated mice. Rather, it potentiated the effect of reserpine in the first 24 hr. This could lead us to doubt a relation between a lack of catecholamines and the analgesic effect of reserpine, or at least to exclude a primary role for them in this phenomenon.

LSD–25 has been demonstrated by Freedman (1961) and Freedman and Giarman (1962) to induce a rise in levels of rat brain 5-HT and to stimulate repletion of 5-HT after reserpine release. If we assume that depletion of 5-HT could play a role in the production of the analgesic effect, we could easily understand the action of LSD–25 in our experiments. Nevertheless, we have to remember that LSD–25 is a potent central stimulant and that we have seen central stimulants to be effective in reducing the analgesic action of reserpine.

The facts mentioned above suggest that 5-HT more than catecholamines would be involved in the mechanism of the analgesic action of reserpine. Depletion of 5-HT after reserpine injection seems to be closely related to this action. This fact does not allow us, however, to postulate that decreased levels of 5-HT in the central nervous system are the cause of analgesia.

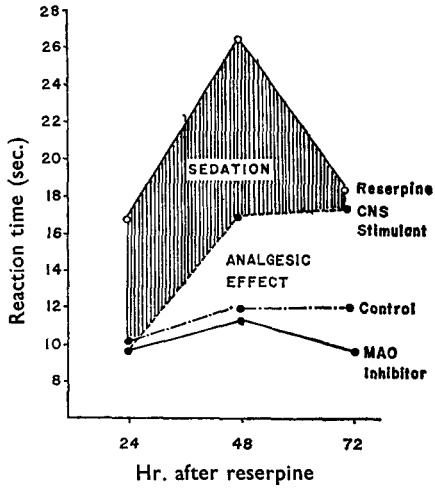

FIG. 1. Theoretical interpretation of the effects of the CNS stimulants and MAO inhibitors upon the RT of animals submitted to a single injection of reserpine. The data are taken from Tables I and II.

Since central nervous system stimulants are able to reduce the RT increased by reserpine, one might assume that part of the effect observed is due to sedation of the animals clearly seen in the first period after the administration of reserpine. As these drugs had much less or no effect at the second and third periods, when reserpine has already been partially or totally eliminated, it might be thought that the effect of the central

nervous system stimulants is confined to a direct antagonism of the sedation produced by reserpine, and therefore has little to do with the more lasting analgesic effect possibly due to a lack of 5-HT in the cerebral structures. A pictorial interpretation of the phenomenon is presented in Fig. 1, in which the shaded zone corresponds to the sedation effect which might be completely counteracted by the central nervous system stimulants (bemegride and ephedrine). Along these lines, the antagonising effect of the inhibitors of MAO (pheniprazine and 2596IS) would extend over both phases of the phenomenon, as it is known that they are also stimulants of the central nervous system. However, with the experimental set up we have used we could not measure the exact contributions of the two phases of the phenomenon to the observed extension of the RT, and the scheme of Fig. 1, only sets the limits beyond which a simple sedation would not be enough to explain the delayed analgesic effect induced by reserpine.

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